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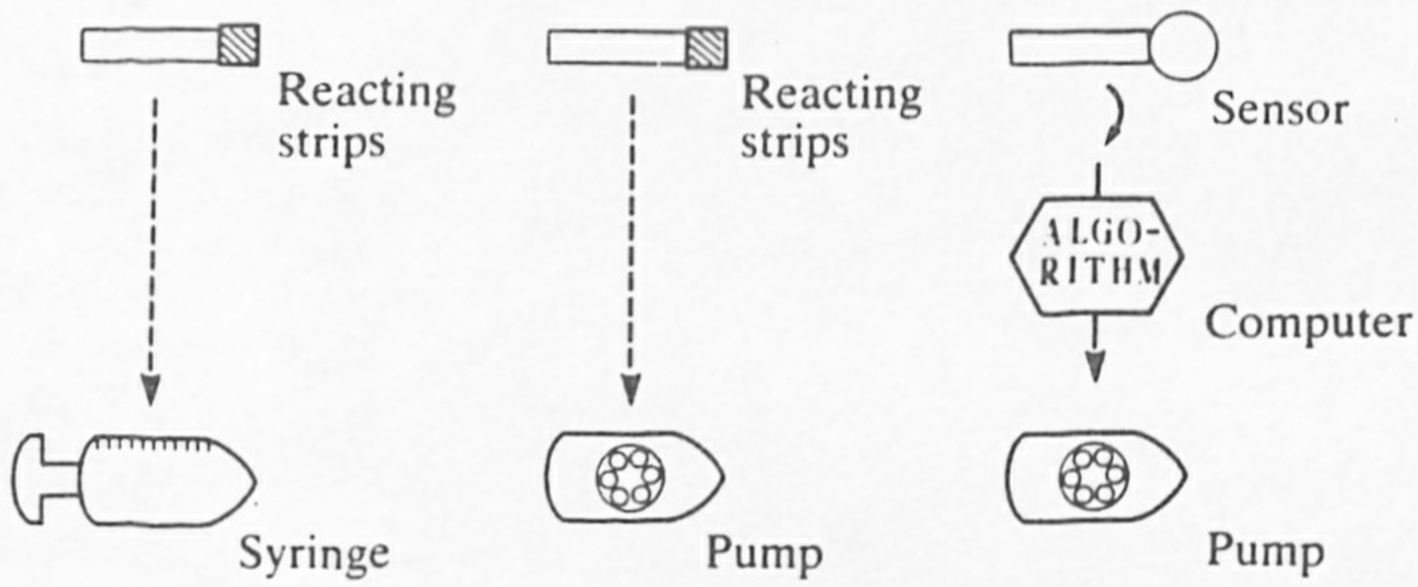
22.1 Introduction

Insulin, a polypeptide hormone produced by the beta-cells of the pancreas, is essential in many metabolic pathways for carbohydrates, proteins, and fats; in the absence of normal insulin secretion, body fuel homeostasis is deranged. Diabetes mellitus is characterized by a relative or an absolute insulin deficiency manifested by loss of control of the circulating blood glucose levels, and by other metabolic abnormalities.

Diabetes is a common disease in affluent societies, affecting from one to three per cent of the population, and often five to ten per cent of those over 40 years of age (Hamman 1983). Where systematic surveys have been performed in the developing nations, rates of one to two per cent of the total population prevail (Bennett 1983). Thus, diabetes is a major worldwide health problem with a great social and economic impact due largely to its later complications. Albisser and Spencer (1982) referring to the Report of the National Commission on Diabetes to the Congress of the United States (1976) suggest that, in that country, diabetics are 25 times more prone to blindness than non-diabetics, 17 times more prone to kidney disease, 5 times more prone to gangrene, twice as prone to heart disease, and have a life expectancy of approximately one-third less than the general population.

Diabetes mellitus is an heterogeneous disease and only a minority of patients, representing however 3 per 1000 of the general population, are so severely insulinopenic as to require insulin therapy. Since its introduction in the early twenties up to the last years of the seventies, insulin therapy was possible only through discontinuous insulin administration, by one, two, or occasionally, several daily insulin injections.

The search for better methods for treating insulin-dependent diabetes and its complications has led to the development of new devices for insulin therapy in the last decade. Infusion systems for continuous insulin delivery (insulin pump), including a reservoir, a pump, and a power supply packed into a portable single unit, have been made available to clinicians and diabetic patients. Efforts to develop a portable self-regulated system, associating an



a. Conventional therapy b. Open-loop system c. Closed-loop system

Fig. 22.1 Scheme of three possible methods of insulin therapy.
(a) Intensive conventional therapy: multiple insulin injections dependent on manual glucose measurement with reacting strips.
(b) Open-loop system: continuous preprogrammed insulin infusion dependent on manual glucose measurement with reacting strips.
(c) Closed-loop system: continuous self-regulated insulin infusion. The glucose level, continuously measured by the sensor, is translated by the computer in a variable rate of insulin delivery.

implantable glucose sensor with the insulin delivery device, are in progress in several laboratories. This system is referred to as a closed-loop system, in contrast to the former, non self-regulated system, known as the open-loop system (Fig. 22.1). The control of insulin delivery by open-loop and closed-loop systems, as compared to the physiological regulation of insulin secretion, is shown in Fig. 22.2.

This chapter will review the advantages of the closed-loop system of insulin therapy, the requirements for an implantable glucose sensor, and the state of present development and applications of glucose sensors.

22.2 Are closed-loop insulin infusion systems really necessary?

The evidence of a relationship between the microvascular complications of diabetes mellitus and hyperglycaemia (Tchobroutsky 1978) led to the intensification of insulin therapy, either by multiple daily insulin injections or continuous insulin infusion, in the hope that it would improve metabolic control and therefore prevent the occurrence of these late complications. Rizza *et al.* (1980) comparing the control of blood sugar by an artificial endocrine pancreas (closed-loop system), continuous subcutaneous insulin infusion (open-loop system), and intensified conventional insulin therapy, in insulin-dependent diabetes, found no significant differences among the three regimens and suggested that all three methods have the potential to achieve a similar near-normalization of glycaemia.

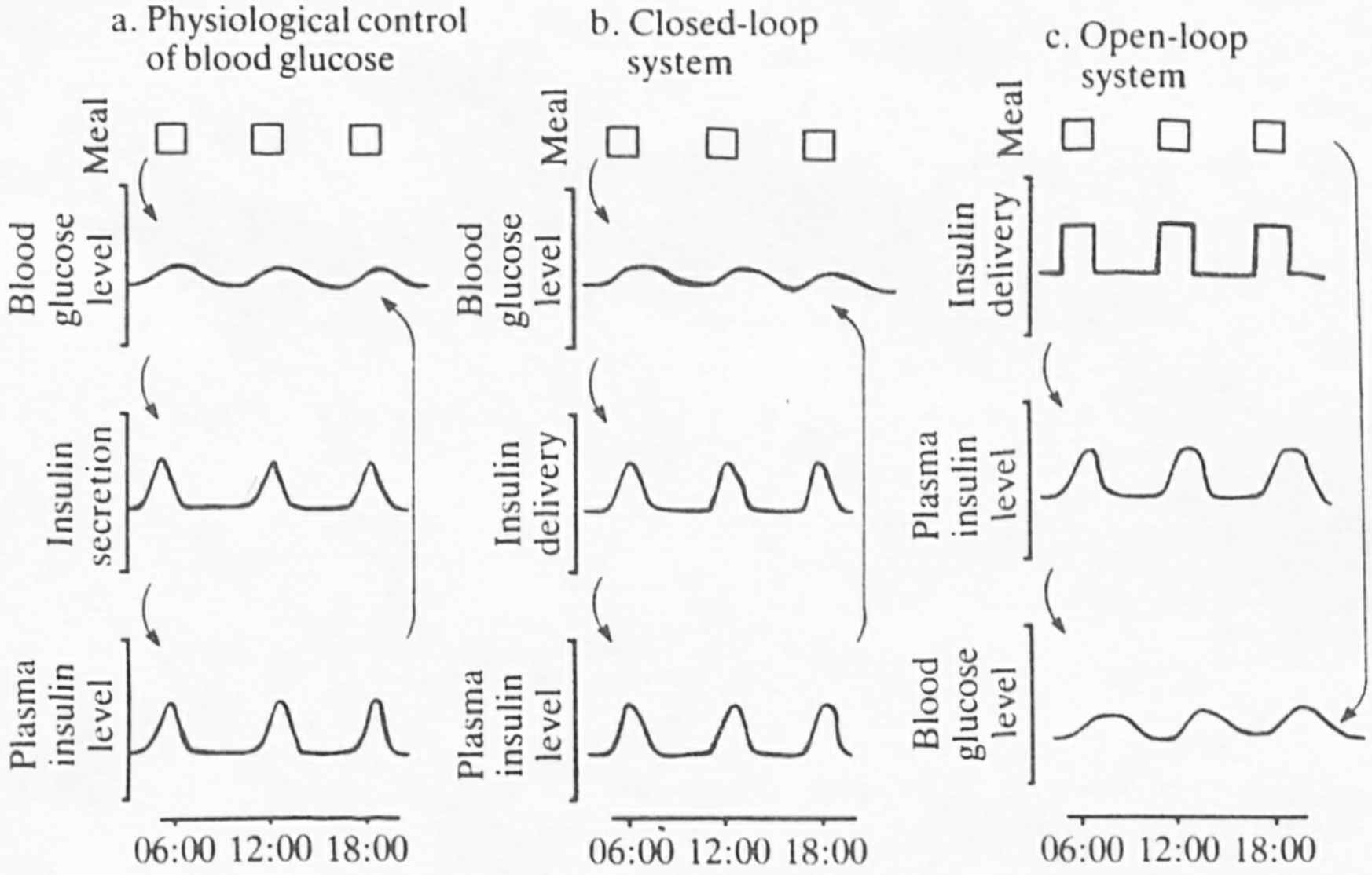


Fig. 22.2 Physiological regulation of blood glucose by the endocrine pancreas (a), control of insulin delivery in closed-loop (b) and open-loop (c) systems. In the open-loop system, insulin delivery is programmed to normalize the blood glucose but it is not regulated by the glucose level.

The main advantages and disadvantages presented by these three methods of insulin therapy are summarized in Table 22.1. Intensive conventional insulin therapy is inexpensive, calls for no special equipment and is immediately available to every patient. However, multiple daily injections of insulin are necessary to achieve a near normal glycaemic control.

Open-loop devices do not have this limitation. Nevertheless, they are expensive, must be carried by the patient, and like any mechanical devices, are subject to malfunction. The problem of insulin aggregation, with loss of biological activity and obstruction of the internal passages of the device has been described (Lougheed *et al.* 1980). Both conventional insulin therapy and open-loop therapy need frequent glucose measurements by the patient to maintain adequate glycaemic control since diet and exercise demand an adaptation of insulin doses. Both therapy methods present, however, further advantages concerning the timing of mealtime insulin bolus and the route of insulin delivery. The mealtime insulin bolus is not controlled by nutrient absorption in the gut, as in normal individuals and in closed-loop therapy (Fig. 22.2), and thus, must be controlled by the patient. As the post-prandial blood glucose and insulin levels are affected by the interval between insulin administration and meal ingestion, this interval, if appropriately chosen, may contribute to the normalization of glycaemia and insulin

Table 22.1 Main advantages and disadvantages of different methods of insulin therapy

	Conventional therapy	Open loop	Closed loop
Disadvantages	Multiple injections	Expensive	Portable device not available
	Frequent glucose measurement	Frequent glucose measurement	Hyperinsulinaemia
		Must be carried by the patient	Venous injection
		Subject to malfunction	Must be carried by the patient
		Insulin aggregation	Subject to malfunction
			Insulin aggregation
Advantages	Immediately available	Immediately available	Independent of external glucose measurements
	Inexpensive: no special equipment	Free of multiple injections	Auto-adaptation to exercise and diet changes
	Subcutaneous injection	Subcutaneous injection	

profile. Dimitriadis and Gerich (1983) compared the effects of 30-min subcutaneous insulin infusions started 60 min, 30 min, and immediately before meal ingestion on postprandial plasma glucose and insulin profiles in subjects with insulin dependent diabetes mellitus. They found that administration of insulin 60 min before meal ingestion provided plasma glucose and insulin profiles closest to normal and permitted less insulin to be used. This anticipation of 60 minutes may be necessary for two complementary reasons: first, part of this time may be required to build up a physiological hepatic insulinization from insulin delivered subcutaneously. Second, insulin secretion in non-diabetic subjects is not controlled only by blood glucose rise: the response of insulin secreting cells is anticipated under the influence of different nerves and of gastroenteric hormones. Thus, if insulin doses and the timing of injection or bolus infusion are carefully chosen, near normal glucose control can be obtained by intensive conventional therapy and open-loop systems through the subcutaneous route of insulin delivery. In that way the complications associated with long-term vascular access for intravenous insulin infusion can be avoided.

The main advantage of a closed-loop insulin delivery device is its independence of external glucose measurements and its ability to cope with the

variations of insulin requirement brought about by exercise and diet. Nevertheless, the glycaemic normalization achieved by these devices is frequently associated with peripheral hyperinsulinaemia (Horwitz, Zeidler, *et al.* 1980). Hyperinsulinaemia is a common feature of any insulin administration through a peripheral route, and is mainly due to the absence of the portal-peripheric insulin gradient. Furthermore hyperinsulinaemia might also be the consequence of the time lag in insulin administration in response to the glucose challenge. Thus, this hyperinsulinaemia can be avoided by the combination of the feedback controlled insulin administration with a pre-programmed preprandial insulin infusion (Calabrese, *et al.* 1982). Therefore, the possibility of a 'manual' or 'semi-automatic' mode should be considered in the design of closed-loop systems.

Currently, closed-loop systems present several disadvantages. They are cumbersome bedside devices that need continuous blood withdrawal from the subject in order to ensure the automatic glucose analysis. A small portable device is not yet commercially available. Closed-loop systems, portable or not, need long term venous access for insulin delivery. Insulin absorption by a subcutaneous or peritoneal route is not fast enough to enable an efficient feedback control of the infusion rate. Finally, closed-loop systems, being automatic devices, should be extremely reliable, both mechanically and in the reading of the glucose sensor, otherwise their main advantage, i.e. less demanding glucose control by the patient, would be lost. Pump-induced insulin aggregation seems to be an additional problem to be solved (Brennan *et al.* 1985).

22.3 Why is a portable closed-loop insulin infusion device not yet available?

Such a device consists essentially of a glucose sensor, a pump and a computer that translates the information provided by the sensor into a variable rate of insulin infusion. The pump and the computer components of the device are commercially available. By contrast, implantable glucose sensors that prove to be reliable are still to be developed.

The great majority of the glucose sensors developed so far operate through the oxidation of β-D-glucose by dissolved oxygen in the presence of β-D-glucose oxidase (GOD EC 1.1.3.4.), according to the following reaction:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

They consist of electrochemical detectors (electrodes) associated in different ways with the enzyme support. The chemical reaction may be monitored via three of its constituents, i.e., oxygen depletion, gluconic acid, or hydrogen peroxide formation.

Table 22.2 Main requirements for a glucose sensor for an artificial beta-cell

High specificity for glucose
Linearity of response from 1 to 15 mmol/l of glucose
Response time less than 10 minutes
Response independent of hydrodynamics and oxygen variations in tissues
Stability of glucose oxidase membrane at 37 °C in tissues
Biocompatibility
Prolonged lifetime (at least several days)
Miniaturization of the sensor head

The requirements to be fullfilled by a portable or implantable glucose oxidase type of sensor for use in a closed-loop device (Thévenot 1982) are described below and summarized in Table 22.2. General requirements, also valid for other types of glucose sensors, are marked with an asterisk.

1) High specificity for glucose*. In the case of glucose oxidase sensors this includes high enzymatic specificity and high electrochemical specificity of associated detectors. The first condition is always valid since glucose oxidase catalyses the oxidation of only very few species besides glucose, and at a much lower rate (Barman 1969). On the contrary, the second condition is often non-valid and depends mainly upon the type of electrochemical detector used (see Section 22.4).

2) Linearity of *in vivo* response from 1 to 15 mmol/l (Fig. 22.3b)*; this rather limited linear range is justified by the recent findings of Harrison *et al.* (1985) who described the properties of isolated human islets of Langerhans (Fig. 22.3a). The threshold concentration of glucose required for stimulation of insulin release was between 2 and 4 mmol/l, insulin secretory response to glucose stimulation had half-maximal values at a glucose concentration of approximately 5 mmol/l and a plateau at 10 mmol/l. Under *in vivo* conditions the calibration curve of a glucose oxidase sensor may not always be linear over this range (example: Fig. 22.4, curve A). In fact, the tissue or blood glucose level, especially in diabetics, may be higher than the apparent Michaelis constant (K_M) of glucose oxidase solutions for glucose in air-saturated solutions, i.e. 4 to 10 mmol/l (Apotheker A., Thévenot D. R., Wilson G. S., unpublished data). However, it is possible to get a calibration curve linear over a much higher concentration range, i.e. up to 20–30 mmol/l, if the glucose flux is reduced by membranes of low permeability to glucose. This may be achieved by an external membrane covering the enzymatic membrane (Fig. 22.4, curve C) or by the enzymatic membrane itself (Fig. 22.4 curve B).

3) Response time less than 10 min*; Sorensen, *et al.* (1982) using a theoretical physiological pharmacokinetic model of glucose homeostasis

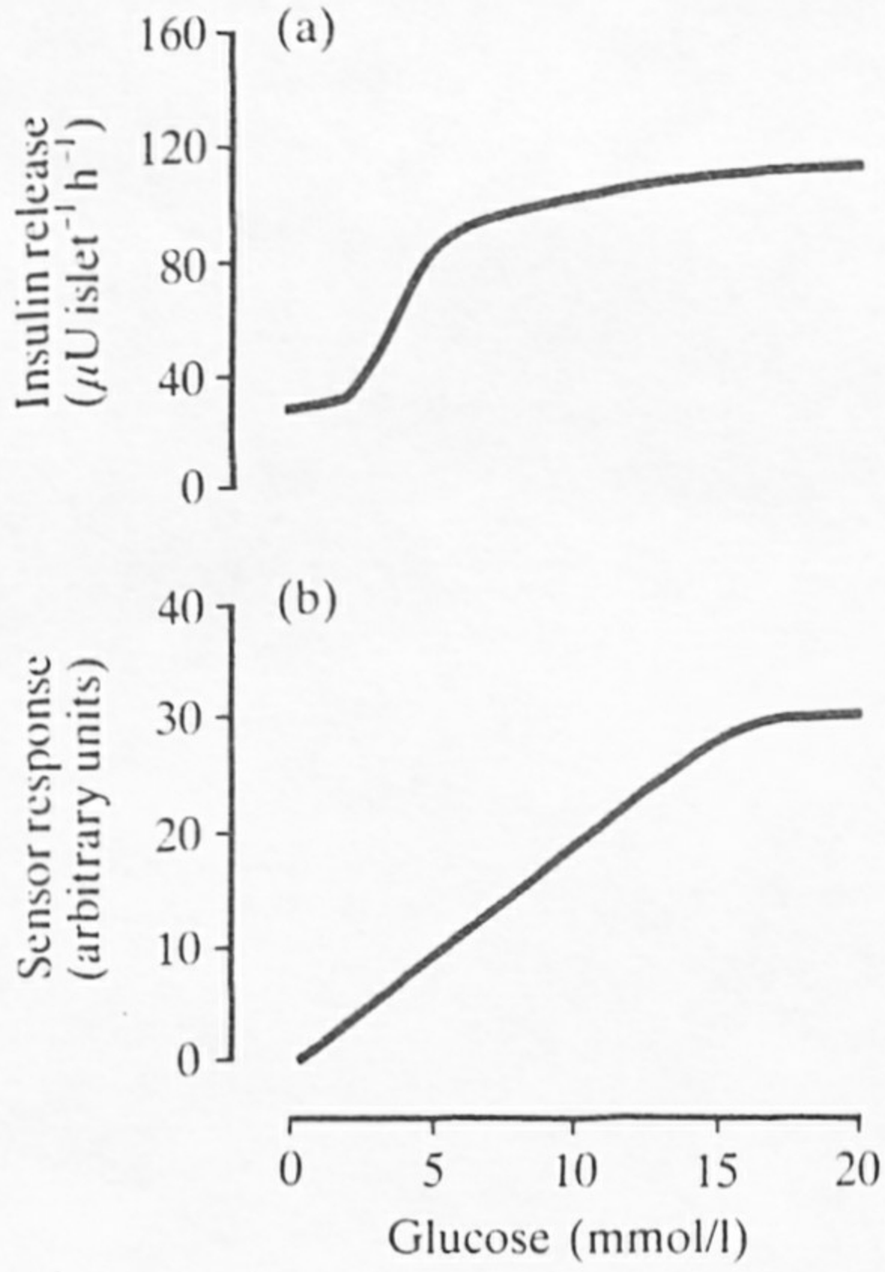


Fig. 22.3 (a) Insulin secretion by isolated islets in respone to glucose (after Harrison *et al.* 1985). Note the sigmoidal relationship and the plateau observed at values higher than 10 mmol/1. (b) Linearity of an implantable glucose sensor for the artificial endocrine pancreas. Due to the response of natural beta cells, the linearity of the sensor response may be limited to 15 mmol/l of glucose.

showed that increases in sensor delay resulted in progressive loss in glucose regulation, exacerbation of hyperinsulinaemia, and increased insulin requirements.

4) Independence of sensor response to fluid hydrodynamics in vessels or tissues*.

5)Independence of sensor response to oxygen level variations in the sensor surroundings and oxygen consumption by the sensor itself. Oxidation of glucose by dissolved oxygen is an irreversible process with a steady state that may be controlled either by the enzymatic oxidation reaction with high temperature dependence (6–10%/°C) or by substrate diffusion with low temperature dependence (2–4%/°C) (Racine and Mindt 1971; Kamin and Wilson 1980). Under such heterogeneous kinetics, the glucose electrode consumes what it is supposed to monitor. This is a characteristic common to Clark's oxygen sensor (see Section 22.4.1.). Whatever the electrochemical detector associated with the glucose oxidase membrane, the stability of its readings is affected by external diffusion (i.e. fluid flow rate near the membrane), internal diffusion (i.e. permeability to substrates), as well as by oxygen concentration level in or near the mem-

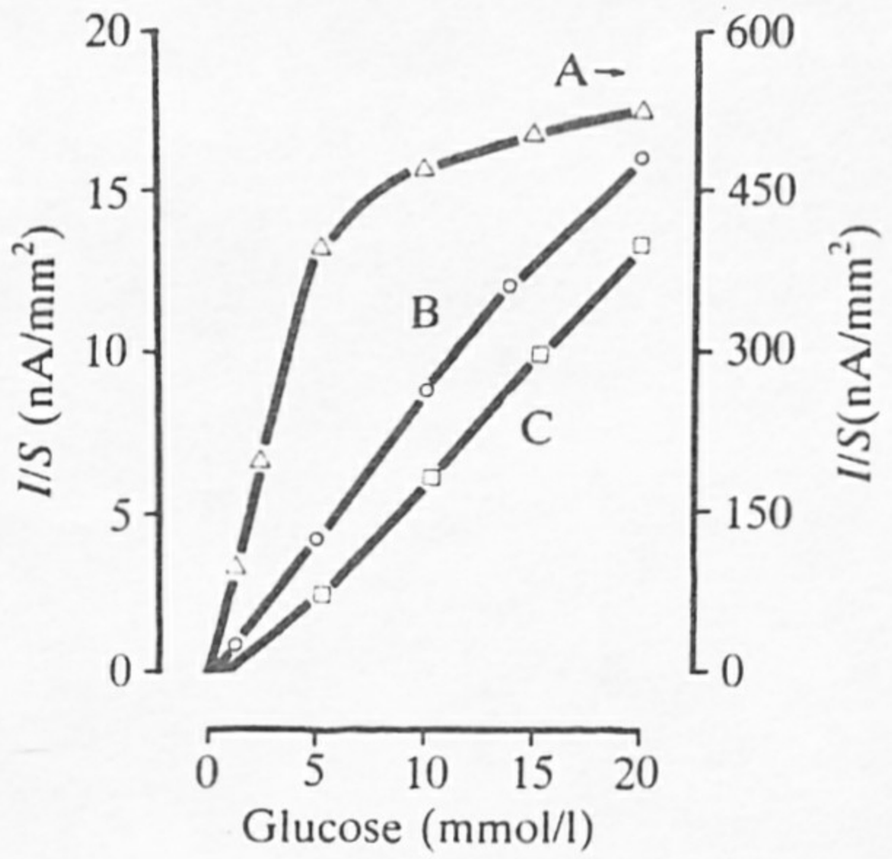


Fig. 22.4 Different types of calibration curves for a glucose sensor using different glucose oxidase membranes (after Sternberg R., Tallagrand T., Thévenot D. R.. Unpublished data): (a) GOD collagen membrane (right-side *Y* axis). (b) GOD cellulose acetate membrane (left-side *Y* axis). (c) GOD collagen membrane covered with a pinholed Teflon membrane (left-side *Y* axis). Note that the use of an additional non-enzymatic membrane or of a cellulose acetate membrane both extends the linear range of the system and impairs its sensitivity.

branes. In an ideal situation these factors should be kept constant. In the case of implantable glucose electrodes these ideal conditions are difficult to obtain. Clark's oxygen detectors that have a cathode diameter approximately equal to the membrane thickness (10–20 microns), are less dependent on hydrodynamics, due to the hemispheric diffusion pattern (in contrast with the linear pattern) obtained under this particular situation (Bard and Faulkner 1980; Wightman 1981). Accessibility of substrates and adequate oxygen level at enzymatic sites can be indirectly controlled by using an additional external membrane more permeable to oxygen than to glucose and/or by using enzymatic layers with a high partition coefficient for oxygen.

6) Long-term mechanical, chemical, and enzymatic stability of glucose oxidase and its support at 37°C, in whole blood, lymph, or tissue.

7) No leaking of glucose oxidase into fluids and tissues surrounding the sensor; being a foreign enzyme, its recognition by the immune system would provoke an immune reaction.

8) Biocompatibility of all implanted parts of the sensor; absence of implant encapsulation by fibroblasts and giant cells*. Woodward (1982) suggested that the optimal configuration for a subcutaneously implantable sensor is in the form of a wire or filament. Such a structure, if measuring less than about 2 mm in diameter, would evoke a minimal tissue response.

9) The scaling down of the sensor should not modify the geometrical, physical, and enzymatic characteristics which control its analytical properties.
10) The system should require minimal calibration and zero adjustment*.
11) Finally, the sensor should have a prolonged lifetime, it should be easily replaceable if necessary and not be expensive if it has to be replaced*. In the case of sensors to be partially inserted in the subcutaneous tissue, in a needle-like fashion, a lifetime of several days, if not weeks, could be accepted. Obviously, a totally implantable device would require a much longer lifetime.

In the remaining sections of this chapter an overview is presented of the significant results obtained in the development of glucose sensors and their application in closed-loop insulin infusion devices.

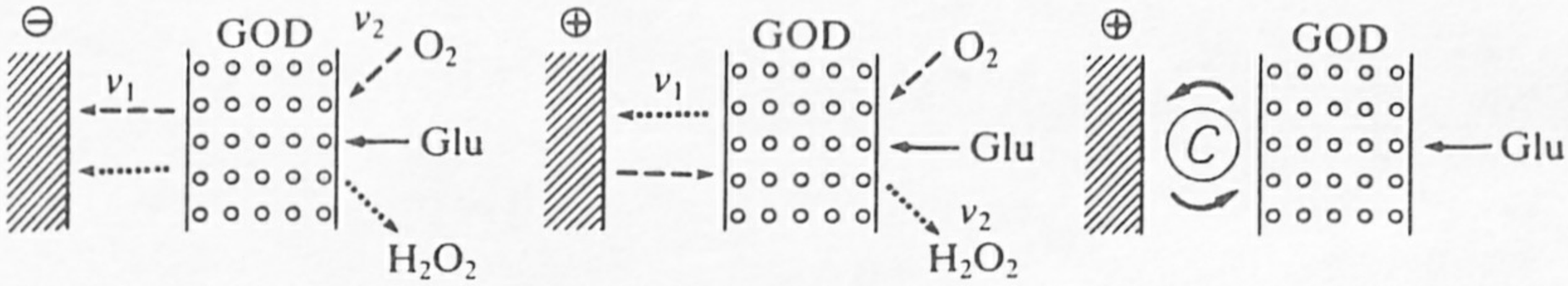
22.4 Glucose oxidase electrochemical sensors for the artificial endocrine pancreas: types of detectors.

22.4.1 Oxygen detectors

Clark and Lyons (1962) described the first specific glucose electrode (Chapter 1). The enzyme was retained on a polymer membrane and an amperometric oxygen electrode estimated the decrease of oxygen as the reaction proceeded. The Clark-type oxygen electrodes are almost insensitive to all types of interfering substances, but they are obviously very sensitive to variations in partial pressure of oxygen within the fluid in contact with the electrode. Thus, misreadings due to physiological or pathological fluctuations of oxygen partial pressure are to be expected under *in vivo* conditions. This problem may be surmounted by the addition of a second electrode, not associated with a glucose oxidase membrane, forming a differential system (Updike and Hicks 1967).

Improvements in this system by Bessmann and Schultz (1973) led to a prototype implantable sensor using two galvanic oxygen electrodes as detector. Oxygen had access to the electrodes through a polypropylene membrane, the external side of which was fastened to a matrix of nylon cloth. Glucose oxidase was covalently bound to the matrix, in the working electrode, by glutaraldehyde. The whole was contained in a plastic disc of 2 cm diameter by 0.25 cm depth. The sensor had a useful *in vivo* lifetime of four days but a less than optimal sensitivity to glucose, due in part to the low oxygen partial pressure in subcutaneous tissues (Bessman *et al.* 1977).

An additional problem with this type of sensor (Fig. 22.5a) is the competition for oxygen between the glucose oxidase membrane (flux v_2) and the oxygen detector itself (flux v_1); if the cathode is not small enough, the latter flux may interfere with the apparent glucose oxidase activity.



$E = -0.4$ V/AgCl

a. Oxygen cathodic detector

$E = +0.6$ V/AgCl

b. Hydrogen peroxide anodic detector

$E = +0.16$ V/AgCl

c. Cofactor detector

Fig. 22.5 Types of detectors used in glucose oxidase electrochemical sensors for artificial endocrine pancreas: (a) oxygen cathodic; (b) hydrogen peroxide anodic; and (c) cofactor detector. See text for explanation.

22.4.2 pH detectors

Glucose sensors based on the detection of gluconic acid via a pH electrode have been developed. Nevertheless they present poor sensitivity, selectivity, and linearity of calibration curves (Nilson *et al.* 1973) and thus cannot be implanted in the highly buffered body fluids.

22.4.3 Hydrogen peroxide amperometric detectors

Amperometric detection of enzymatically generated hydrogen peroxide is probably the most developed type of glucose sensor (Guilbaut and Lubrano 1973; Scheller *et al.* 1977; Thévenot *et al.* 1978) (Fig. 22.5b). Clemens *et al.* (1977) adapted one of such sensors for use in a bedside-type artificial pancreas. Similar sensors have been adapted for the same purpose by several groups (see Section 22.7). Over the last ten years, improvements have been made in the sensor design, the binding of the enzyme to its support, and the functional characteristics of the electrodes.

This type of detector is very sensitive to glucose; its lowest detection limit may reach 10 nmol/l (Thévenot *et al.* 1978). Hydrogen peroxide amperometric detection is also very sensitive to naturally occurring electron donors, such as ascorbate, urate, and tyrosine. Methods have been developed to increase the selectivity of the glucose electrode towards such interfering substances. Either the response is compensated by a non-enzymatic detector (Thévenot *et al.* 1978) or the platinum anode is covered by selectively impermeable membranes (cellulose acetate, for instance) with pores that will exclude ascorbate and most other potential interfering substances (Yellow Springs Instrument Co. 1975).

The independence on oxygen concentration of hydrogen peroxide detection is an advantage in sensor design. Nevertheless the local oxygen level

necessary for the enzymatic reaction to occur must be taken into account: in that way, membrane partition and diffusion coefficient for oxygen play an important role in glucose response patterns. Oxygen is regenerated during the electrochemical oxidation of hydrogen peroxide on the platinum surface according to the reaction:

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

Optimization of the collection efficiency of the detector (Fig. 22.5b) i.e., the ratio between the part of the enzymatically generated hydrogen peroxide flux oxidized on the platinum (v_1) and the total flux ($v_1 + v_2$), v_2 being the part of the flux diffusing towards the bulk solution, would result in a greater availability of oxygen in the enzymatic layer and in a greater independence of oxygen diffusion from the bathing fluids, once the reaction had started (Coulet *et al.* 1980).

Finally, hydrogen peroxide anodic oxidation is not always diffusion controlled and its rate may limit the signal from the sensor. This rate may depend on electrode conditioning (Dubois 1984).

22.4.4 Hydrogen peroxide potentiometric detection

Potentiometric measurement of glucose concentration is the principle of a sensor developed by Schiller, Wingard, and Liu (1982; Chapter 10). Glucose oxidase is immobilized directly on the platinum surface of the working electrode by methods including entrapment in polyacrylamide gel, cross-linking in an albumin matrix with glutaraldehyde, and coupling to platinum through gamma-aminopropyltriethoxy silane (Wingard *et al.* 1979).

In contrast to amperometric detections in which an external potential is applied between the electrodes, and in which oxygen or hydrogen peroxide local concentrations are directly monitored through the generated current, potentiometric detection measures a pseudo-equilibrium potential inside the system. The electrochemcial reaction responsible for this potential appears to result from the interactions between the enzymatically generated hydrogen peroxide and the platinum surface (Wingard *et al.* 1982). The electrode cleaning procedure is always critical to the functioning of the system. Linearity of response, as the logarithm of glucose concentration, was achieved in *in vitro* studies over the range of about 0.6 to 22 mmol/l. Theoretical advantages of this system for *in vivo* utilization, due to the low potential generated, include minimal electrochemical interference and the possibility of micro-miniaturization of the electrode.

22.4.5 Cofactor detectors

The concept of cofactor detectors is based on the ability of cofactors to act as temporary acceptors of the protons and electrons released during the oxidation of substrates by oxidation-reduction enzymes (Chapter 15). The general

idea is to have a solid state type electrode in which a naturally-occurring or an artificial cofactor is an integral part of the electron conducting support and the enzyme is immobilized with the cofactor. The electrode, as a whole, behaves as a cofactor, i.e. an electron acceptor or donor for an enzymatic reaction (Fig. 22.5c).

The coupling of riboflavin to solid carbon, forming a solid state pathway for easy electron transfer, has been described (Wingard 1982). Subsequent developments (Wingard 1983*a*) included the conversion of immobilized riboflavin to FAD and the appearence of enzymatic activity on the addition of the apoenzyme of glucose oxidase. Later, Cass *et al.* (1984) used entrapped ferrocene derivatives, as ferricinium ions, which may be electrochemically oxidized and react with reduced glucose oxidase. If such reagents are present in sufficient excess, then the supply of oxygen to the catalytic layer would have little effect on the enzymatic rate (see Chapters 15 and 16). Recently, Ikeda *et al.* (1985) described a glucose sensor using benzoquinone as a cofactor. Glucose oxidase was immobilized on the surface of a *p*-benzoquinone-carbon paste electrode by coating the enzyme-loaded surface with a nitrocellulose film. Properties of the sensor include the electrocatalytic oxidation of glucose with a linear range up to 15 mmol/l, the response time of about 20 seconds and the insensitivity to variations of oxygen tension in sample solutions.

22.5 Designs of *in vivo* glucose oxidase sensors

The latest developments towards an implantable glucose sensor have favoured three types of sensor design: the plane-geometry type, the vessel-shaped, and the needle type. Plane geometry sensors consist basically of a plane surface support containing the metal working electrode and the reference and counter electrodes coated by various combinations of enzymatic and non-enzymatic, hydrophilic and hydrophobic membranes. The membranes provide a support for the enzyme, an environment for the chemical reaction, and a diffusion barrier assuring the optimal concentrations of glucose and oxygen in this environment. Fischer and Abel (1982) described a plane-geometry sensor mounted into a flow chamber. It consisted of a platinum anode for the measurement of hydrogen peroxide, a silver/silver chloride reference and counter electrode, glucose oxidase immobilized onto sepharose and held by hydrophilic cellulose acetate membranes, and an hydrophobic perforated Teflon membrane in front of the anode. *In vivo* tests using normal and diabetic dogs showed reasonable correlation between the sensor output and the plasma glucose reference values with a response time between 90 and 120 seconds. The linear range for *in vitro* calibration was up to 40 mmol/l of glucose.

An original approach was described by Kondo *et al.* (1981): the sensor is a

vessel-shaped device through which the blood flows. Oxygen-type electrodes and membranes are disposed around its wall. The sensor is introduced into the circulatory system in a fashion similar to an external arterio-venous shunt for hemodialysis. The linear range is up to 16 mmol/l of glucose and the response time is about 10 minutes.

The needle-type sensors are usually micro-electrodes having a platinum core (anode) isolated from an external silver/silver chloride cathode reference and counter-electrode. The electrode is coated with glucose oxidase immobilized in a solution of a matrix material (cellulose diacetate, for instance) in a volatile solvent (acetone, for instance). Shichiri, *et al.* (1982; Chapter 23) have described a subcutaneously implantable needle-type sensor having an *in vivo* response time of 2 to 5 min and a linear response of up to 27 mmol/l of glucose (see Section 22.7).

22.6 Glucose sensors: possible alternative approaches

Glucose sensors based on non-enzymatic approaches have been known for many years. Although they purport to avoid the difficulties associated with heterogeneous enzyme kinetics none of these systems is presently sufficiently developed to permit *in vivo* implantation.

The characteristics of direct electrochemical sensors, consisting of platinum electrodes not associated with glucose oxidase have been studied (Soeldner *et al.* 1973; Gebhardt *et al.* 1978; Richter, *et al.* 1982). The signal is generated by direct glucose oxidation at the anodic surface of a platinum electrode, in response to alternate anodic and cathodic potentials. Their specificity to glucose, in biological fluids, is still less than optimal, due to the interference of endogenous oxidizable substances such as amino acids, urea, ascorbic acid, and of exogenous substances such as alcohol and several drugs. The selection of adequate working potentials and the use of an external selective membrane brings real improvement to the system specificity. An additional problem with this type of detector is the poisoning of the platinum surface by adsorption of gluconic acid and amino acids, which leads to the gradual deactivation of the anode catalyst and inhibition of further oxidation. The deactivation can be offset with regeneration of the working electrode by repeated surface oxidation by electrochemical pulsing. Nevertheless, oxidized radicals are generated and desorbed from the electrode surface together with products of electrode degradation. The present status of the electrocatalytic glucose sensor does not favour its use as an implantable device.

The competition of glucose and fluoresceine-labelled polydextran for the binding sites of the protein concanavalin A, immobilized on the inside surface of a hollow dialysis fibre, is the principle of a sensor developed by Schultz, *et al.* (1982; Chapter 32). This affinity sensor is completed by an

optical fibre inserted in the lumen of the dialysis fibre that allows the measuring of the unbound labelled dextran. This approach presents an advantage, compared to glucose oxidase sensors: the response is determined by the competitive equilibrium between glucose and the signal producing ligand. Thus, kinetics of enzyme reactions and electrode fouling do not affect the magnitude of the sensor response. Optimal specificity and sensitivity could be obtained by the selection of appropriate binding protein and competitive ligand; specific antibodies could be used, for instance. The sensor still suffers from limited stability and relatively long response times when employed as an *in vivo* sensor.

The concept of non-invasive glucose monitoring of the aqueous humor of the eye, by the measurement of the degree of optical rotation produced by the local concentration of glucose, has been advanced by March *et al.* (1979). The requirement of heavy optical equipment is an important drawback in terms of its development into a portable device.

Several endogenous enzymes that use glucose as the primary substrate might be utilized in an enzymatic glucose sensor. They include glucose dehydrogenase, glucokinase, glucose-6-phosphatase and glucose-isomerase (Wingard 1983*b*). In the case of glucose dehydrogenase NAD^+/NADH concentrations could be monitored using a miniature fibre optic spectrometer. At the present time this system is still a theoretical speculation.

The last part of this chapter will deal will the glucose sensor as a part of a closed-loop insulin infusion system. The main characteristics of some implantable sensors are described in Table 22.3.

Table 22.3 Main characteristics of some implantable glucose sensors

	Authors			
	Bessman	Fischer	Kondo	Shichiri
Type of detector	Galvanic cell	Pt anode/ H_2O_2	O_2 (Clark)	Pt anode/H_2O_2
Enzymatic membrane material	Nylon	Sepharose	Nylon	Cellulose acetate
Immobilization procedure	Covalently bound by glutaraldehyde	Covalently bound by cyanogen bromide	Covalently bound by glutaraldehyde	Covalently bound by glutaraldehyde
Non-ezymatic (1) membrane material	Polypropylene	Cellulose acetate	Polypropylene	Polyurethane
Non-enzymatic (2) membrane material		Perforated Teflon	Perforated Teflon	Polyvinyl-alcohol
Sensor geometry	Plane geometry	Plane geometry	Vessel-shaped	Needle-shaped

22.7 The artificial beta cell

The earliest external electromechanical device used as a closed-loop insulin infusion system was described by Kadish (1964). Whenever the blood glucose exceeded 1.5 g/l (8.33 mmol/l) or fell under 0.5 g/l (2.77 mmol/l), insulin or glucagon, respectively, were infused. However this on–off system was not able to normalize the glycaemia. A resurgence of interest in the seventies for this bedside instrument, which became known as artificial beta-cell, led to the refining of the feed-back controlled systems commanding the insulin delivery. Kadish's device was improved by Albisser *et al.* (1974) who subjected the control of insulin delivery to a computer calculated predicted value based on the minute–to–minute variations of blood sugar. Clemens *et al.* (1977) constructed the first of these devices to be commercially available. It was named Biostator Glucose-Controlled Insulin Infusion System (60 kg, $42 \times 46 \times 46$ cm). A number of similar artificial beta cells, using extra-corporeal glucose sensors, have been since then fabricated and evaluated by several groups, including Mirouze *et al.* (1977), Slama *et al.* (1977), Kraegen *et al.* (1979), Goriya, *et al.* (1979) and Fischer, *et al.* (1980).

Bessman *et al.* (1977) reported the implantation into a diabetic dog of a small artificial beta cell consisting of an oxygen-detector glucose sensor, electronics, a micro pump, and a power supply. The sensor was similar to the one previously described in Section 22.4.1. The pump was a piezoelectric device separated from the insulin reservoir by a solenoid valve. Insulin was delivered into the peritoneal cavity when appropriately phased pulses were applied to the pump and valve. However, in this experiment, as well as in the observations on seven additional dogs (Bessman *et al.* 1981), the amount of insulin administered to the dogs was clearly insufficient, due to the inadequate response of the glucose sensor to the glucose levels.

A remarkable achievement in terms of miniaturization was reported by Shichiri *et al.* (1982) who developed a wearable closed-loop device (400 g, $12 \times 15 \times 6$ cm) associated with an implantable needle-type sensor. Short term glycaemic control was achieved in diabetic patients connected to the instrument (Shichiri *et al.* 1984). These results are presented in Chapter 23 of this book.

22.8 Conclusion

Our understanding of the physiological, physicochemical, and electrochemical mechanisms underlying the basic requirements for an *in vivo* glucose sensor has expanded in recent years. The fruits of this understanding, in terms of technology, are beginning to be available. However, several questions remain unanswered and several answers are still not translated into practice.

Concerning the sensor functioning under conditions of *in vivo* implantation, the optimal arrangement of the glucose oxidase support and the protective membranes has still to be found, allowing long term enzymatic stability and adequate glucose and oxygen local concentrations with minimal tissue reaction. A better understanding of the operational properties of such sensors, both *in vitro* and *in vivo*, would allow their design and performance to be optimized.

Other approaches than the glucose oxidase sensor may prove to be worthwhile. The affinity type of sensor could be a promising alternative. Implantable sensors usually require a membrane barrier between the sensing element and the biological fluid. It is clear that the failure of such membranes to maintain reproducible analyte transport characteristics is a major cause of biosensor malfunction.

Finally, the expectations aroused by the development of a reliable sensor for long term use in a portable closed-loop insulin infusion system justify the efforts being made in ongoing studies. More easily attainable good glycaemic control in diabetic subjects could, hopefully, prove to be a major step in the prevention of the late complications of diabetes.

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